Periodicity in recA protein-DNA complexes

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Abstract The reaction of guanine residues with dimethylsulfate was studied for complexes of recA protein with fluorescent dye tagged double stranded oligonucleotides. The patterns of dimethylsulfate modification obtained demonstrate a similarity of DNA states in the complexes with recA protein formed as a result of recA promoted strand exchange and renaturation reactions. The guanine modification efficiency varies periodically as a function of the base position along the oligonucleotide axis, with a period of 3 nucleotides. This effect suggests that the arrangement of recA monomers along the oligonucleotide is strictly ordered, and the dimethylsulfate reactivity of a guanine residue depends on the site of its binding in a recA monomer.

Key words: RecA protein; Fluorescent dye-labeled oligonucleotide; DNA chemical modification

1. Introduction

The RecA protein from *Escherichia coli* catalyzes the central stages of the general genetic recombination – DNA synapsis and strand exchange (for review see [1]). The first step of the recA protein promoted strand exchange reaction in vitro is cooperative binding of recA protein with ss-DNA to form a helical presynaptic nucleoprotein filament. This complex is able to anneal with a complementary ss-DNA or displace a homologous strand from ds-DNA substrate resulting in formation of stable recA protein complexes with heteroduplex DNAs. A stable complex of recA protein with ds-DNA is also formed when ds-DNA is co-incubated with recA protein in the presence of ATP-S cofactor. The exact structural relationship of these differently formed complexes remains to be elucidated.

For structural studies and practical applications of the recA protein system it is important to obtain stable stoichiometric complexes of recA protein with oligonucleotides of a minimum length. However, the binding of recA protein with short oligonucleotides is rather ineffective, complete oligonucleotide binding being achieved under excess of the protein [2]. In the previous paper [3] we demonstrated that recA protein-oligonucleotide binding efficiency increased when fluorescent dye-labeled oligonucleotides were used as the binding substrates; we also demonstrated the strand exchange reaction in this system and described different procedures for obtaining stable recA-oligonucleotide complexes.

In the present paper DNA chemical modification was used for further characterization of the structural organization of the recA protein complexes with dye tagged oligonucleotides and comparison of the complexes formed using different procedures.

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Abbreviations: ATP-S, adenosine-5′-*O*-(3-thiotriphosphate); TAMRA, tetramethylrhodamine; FAM, 5′-carboxyfluorescein; PAG, polyacrylamide gel; DMS, dimethylsulfate; ss and ds, single- and double-stranded, respectively; TEA, triethanol acetate buffer

2. Materials and methods

RecA protein was purified and dye tagged oligonucleotides were synthesized as described [3]. A fluorescent dye molecule with a 6 atom spacer was introduced at the oligonucleotide 5′-end using the Aminolink-2 system (Applied Biosystems) as described in the manufacturer's protocol. The synthesized oligonucleotides are listed in Table 1.

RecA protein was complexed with the oligonucleotides in the presence of ATP-S as described [3] except that TEA buffer was used instead of Tris. The general outline of different approaches used for the complexes formation is presented in Fig. 1. The complexes formed were chilled on ice, and DMS was added to 1% final concentration. After 30 min incubation at 0°C the reaction was stopped by addition of mercaptoethanol and SDS followed by ethanol precipitation and then the product was treated according to the 'G greater than A' method of Maxam and Gilbert [4].

The reaction products were analyzed by electrophoresis on 16% denaturing PAG with a DNA sequencer ABI 370A. Electrophoretic profiles were normalized to signal amplitudes for unmodified oligonucleotides.

3. Results

DMS reacts with N7 of guanine residues exposed in the major groove of ds-DNA and, to a lesser extent, with N3 of adenine residues in the minor groove. Fig. 2 presents the results of DMS treatment of oligonucleotides free in solution and complexed with recA protein. The complexes were formed using 3 different procedures outlined in Fig. 1. As follows from Fig. 2, the reactivity of G residues towards DMS was increased irrespective of the procedure for complex formation. However, the increase was more significant for complexes #2 and #3. Moreover, the efficiency of modification of the G-residues in the complexes varied. In all cases G1 and G2 (for designations see Fig. 2) residues were more reactive towards DMS than G3 residues. To elucidate the difference, dye tagged ss oligonucleotides of different lengths but with the same nucleotide sequence at the 3′-end (see Table 1 and Fig. 3) were used for complex formation.

In the series of these oligonucleotides the distance of each G

Table 1
A list of dye tagged oligonucleotides used for complex formation

Designation	Sequence and dye type
1. 17-mer	FAM-GTAAAACGACGGCCAGT-3′
2. 18-mer	FAM-TGTAAAACGACGGCCAGT-3′
3. 19-mer	FAM-ATGTAAAACGACGGCCAGT-3′
4. 20-mer	FAM-GATGTAAAACGACGGCCAGT-3′
5. 21-mer	FAM-CGATGTAAAACGACGGCCAGT-3′
6. TAMRA-3′	5′-ACTGGCCGTCGTTTTACA-TAMRA

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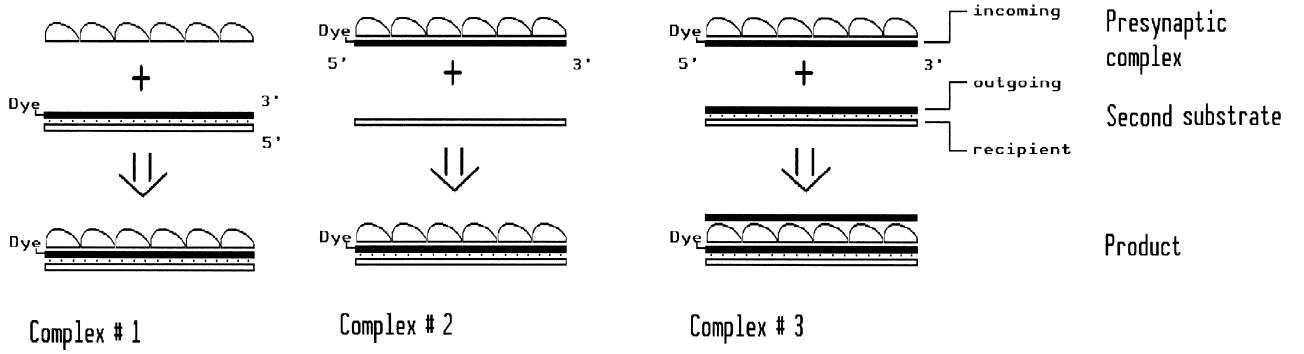

Fig. 1. Reaction schemes and terminology for three modes of recA DNA binding. To form the presynaptic complex recA protein was incubated with a corresponding oligonucleotide, then the second substrate for strand annealing (complex #2) or strand exchange (complex #3) reaction was added and incubation was continued. Complex #1 was formed via immediate co-incubation of the recA protein with ds dye tagged oligonucleotide. Filled and empty bars represent a ss oligonucleotide and a complementary oligonucleotide, respectively. 'Dye' denotes fluorescent dye molecule. In the case of the strand exchange reaction (complex #3) the outgoing strand was demonstrated to be weakly bound to this complex [3].

residue from the 5′-end was different with an increment of 1 nucleotide whereas the surrounding primary structure was conserved. Fig. 4 shows that the reactivity of guanine residues towards DMS is a periodic function of the distance of the G-residues from the 5′-end. In Fig. 4b the result of DMS modification of a ds 18-mer complexed with recA (see also Fig. 2c) is presented. Oligonucleotide lengthening by one base had only limited effect on the modification pattern (Fig. 4c) while lengthening by two bases resulted in the inversion of relative reactivities of adjacent G2 and G3 residues (Fig. 4d). The addition of another base (Fig. 4e) once more inverted the G2 and G3 reactivities thus making the modification pattern

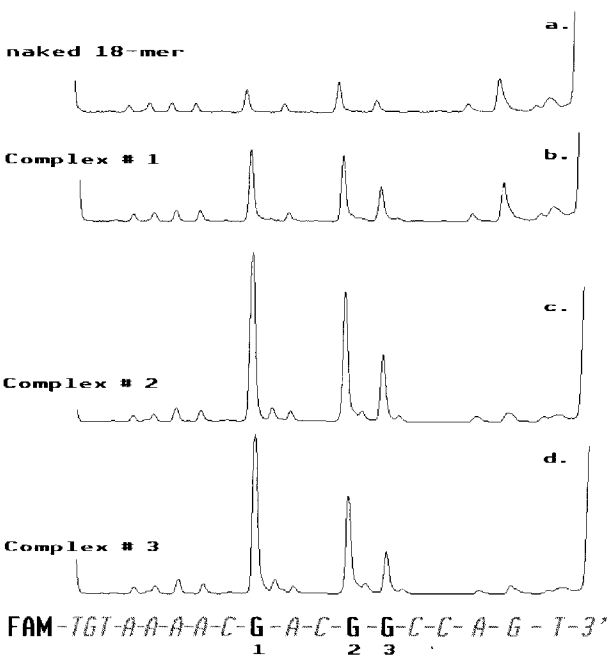

Fig. 2. Methylation of guanines in different recA-oligonucleotide complexes. Modification patterns for the cases of naked oligonucleotide (a), complex #1 (b), complex #2 (c) and complex #3 (d) are presented. The procedures for the complexes formation are outlined in Fig. 1. The sequence of dye tagged oligonucleotides is shown below the electrophoretic profiles.

similar to that presented in Fig. 4b. Shortening of the 18-mer by one base also resulted in inversion of the relative reactivities of G2 and G3 (Fig. 4a), the modification pattern being of type shown in Fig. 4d.

Hence, the gradual oligonucleotide lengthening as described above led to periodic changes in the DMS modification pattern with a period of 3 nucleotides. Accordingly, G1 and G2 residues separated by 2 nucleotides were always characterized by similar reactivity changes (see Fig. 3).

To examine the DMS reactivity of guanines in a recipient oligonucleotide strand (see Fig. 1), the presynaptic complex was prepared as above with 5′-FAM labeled 18-mer whereas a 3′-TAMRA labeled oligonucleotide was used as the recipient strand. Multiwavelength detection of electrophoretic profiles was performed followed by calculation of individual profiles for both dyes. The results are presented in Fig. 5. It can be seen that recA did affect the reactivity of guanine residues in the incoming strand (compare Fig. 2a,c) whereas modification patterns of the recipient strand were the same irrespective of the recA presence. The modification patterns of recipient strands were also unchanged on variation of length of incoming oligonucleotides (an experiment such as presented in Fig. 4) while the modification patterns of the incoming strands were reproducibly changed (not shown).

4. Discussion

An increase in guanine reactivity towards DMS was demonstrated earlier for the complexes formed by direct incubation of recA protein with long ds-DNA molecules [5] (the mode of binding corresponding to complex #1 in Fig. 1). The increase in guanine reactivity reported here for recA complexed with dye tagged short oligonucleotides is in line with this result and suggests that an extreme shortening of DNA substrate and use of dye molecules to stimulate recA protein binding does not crucially change the character of recA-DNA interaction.

Stable complexes of the recA protein with dye tagged oligonucleotides are also formed in the processes of recA promoted DNA strand exchange and DNA renaturation reactions (complexes #2 and #3, Fig. 1). The modification patterns obtained for these complexes (Fig. 2) also reveal an

Fig. 3. Possible arrangements of recA monomers in the complexes depending on oligonucleotide lengths. Each recA monomer contains three sites for binding of individual nucleotides marked as 1, 2 and 3. RecA binding starts at the 5'-oligonucleotide end, each base bound to the 1st, 2nd or 3rd site as dictated by the base position along the oligonucleotide.

increase in the guanines reactivities towards DMS and close overall patterns of the reactivity that imply a structural similarity of these complexes.

The periodicity of the guanine reactivity variation agrees with the features of recA-DNA complex formation known to proceed as a successive binding of recA monomers starting from the ss-DNA 5'-end, each recA monomer covering three nucleotides [1]. The present data indicate that this binding initiates at an exactly determined position at the oligonucleotide 5'-end that results in a strictly ordered (phased) arrangement of recA monomers along the oligonucleotide chain. The position of an individual nucleotide residue relative to an adjacent recA monomer appears to depend on the oligonucleotide length. RecA-DNA binding stoichiometry suggests three possible sites of individual nucleotide binding for each recA monomer (see Fig. 3). Our data demonstrate that the DMS reactivity of a guanine residue depends on the site of its binding.

Moreover, in agreement with the previously reported data of K. Adzuma [6], we demonstrate that complementary DNA strands in the complexes are characterized by different guanine reactivities.

The most probable explanation for enhanced DMS reactivity of guanines in DNA-protein complexes is close proximity of protein groups affecting the modification with DMS, e.g. hydrophobic clusters or so-called lipophilic pockets that facilitate increased local DMS concentrations [7]. If this explanation is true in our case, the present data indicate that the protein hydrophobic moieties are clustered in some restricted regions of the complexes near the incoming DNA strand.

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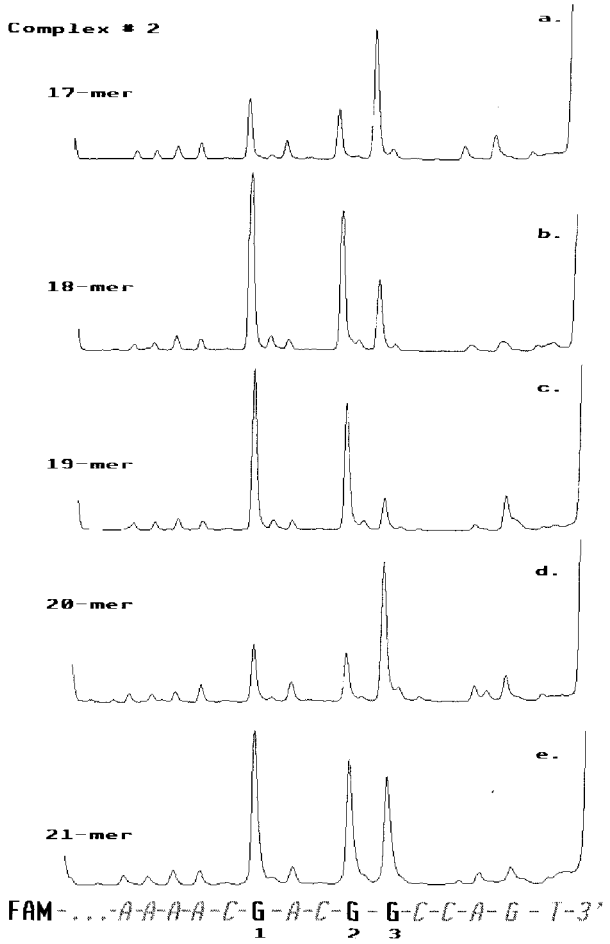

Fig. 4. Methylation of guanines in oligonucleotides of different lengths complexed with recA protein (see also Fig. 3). The lengths of the oligonucleotides used for presynaptic complex formation were: 17 bases (a); 18 bases (b); 19 bases (c); 20 bases (d); 21 bases (e). The profiles were aligned with respect to signals from corresponding G residues.

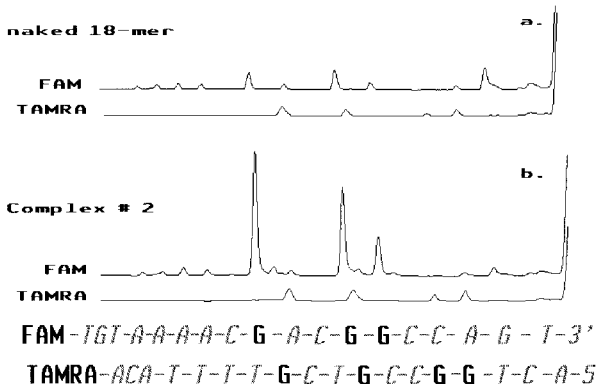

Fig. 5. Modification of a recipient oligonucleotide. Incoming and recipient strands labeled with FAM and TAMRA dyes, respectively, were used for complex #2 formation. The sequences of the oligonucleotides are shown below the profiles. They are aligned to corresponding electrophoretic profiles and therefore shifted relative to each other due to some differences in electrophoretic mobility of the oligonucleotides labeled with different dyes.

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